

to take into consideration the deleted figures. In addition, because no amino acid or nucleotide sequences are present in the specification or drawings, as currently amended, there is no longer a requirement for a Sequence Listing.

Claims 35, 43, and 51 have been amended to recite “RNA of a selected organism” rather than “target nucleic acid.” The amendment is made to harmonize the language of the claims. No new matter is added and no change in scope is intended.

#### **I. No New Matter Is Added to the Claims**

Claims 52-67 have been added with a proviso stating that the molecular interaction site of the claims does not include the 3’ untranslated region of the histone mRNA. The Advisory Action alleges that the phrase “the 3’ untranslated region of the histone mRNA” may raise an issue of new matter “because this phrase can not [sic] found in the specification.” In rejecting a claim under the first paragraph of 35 U.S.C. § 112 for lack of adequate written description, it is incumbent upon the examiner to establish that the originally-filed disclosure would not have reasonably conveyed to one having ordinary skill in the art that an applicant had possession of the now claimed subject matter. *Wang Laboratories, Inc. v. Toshiba Corp.*, 26 U.S.P.Q.2d 1767 (Fed. Cir. 1993). Adequate description under the first paragraph **does not** require literal support for the claimed invention. *In re Herschler*, 200 U.S.P.Q. 711 (C.C.P.A. 1979); *In re Edwards*, 196 U.S.P.Q. 465 (C.C.P.A. 1978); and *In re Wertheim*, 191 U.S.P.Q. 90 (C.C.P.A. 1976). Rather, it is sufficient if the originally-filed disclosure would have conveyed to one having ordinary skill in the art that an applicant had possession of the **concept** of what is claimed. *In re Anderson*, 176 U.S.P.Q. 331 (C.C.P.A. 1973).

Applicants’ specification conveys such possession. Applicants teach, for instance, in Example 3 at pages 37-38 of the specification, use of the known structure in “the 3’ untranslated region of the histone mRNA” to validate Applicants’ methods and strategies. Thus, one having ordinary skill in the art would have reasonably known that an oligonucleotide comprising the 3’ untranslated region of the histone mRNA was part of the prior art. In addition, one having ordinary skill in the art would also have known that, given the description of 3’ untranslated region of the histone mRNA in the prior art, Applicants’ claimed invention did not include the 3’ untranslated

region of the histone mRNA. Thus, Applicants maintain that the originally-filed disclosure would have conveyed to one having ordinary skill in the art that Applicants had possession of the concept of what is now claimed.

The Office Action cites *In re Grasselli* which is purported to support the position taken in the Office Action. The facts in *Grasselli* appear, however, to be quite different than the facts in the present case. In *Grasselli*, the applicants' originally-filed specification appears to have disclosed a catalyst comprising particular elements and/or metals. A negative limitation (*i.e.*, "said catalyst being free of uranium and the combination of vanadium and phosphorous") was added to a claim to remove at least one prior art reference. The originally-filed application, however, appears to have not expressly recited either uranium or the combination of vanadium and phosphorous, let alone their inclusion or exclusion. Thus, the Board in *Grasselli* held that the addition of the negative limitation, under these circumstances, was not adequately supported by the originally-filed specification.

Thus, contrary to the Advisory Action's assertion, the phrase "the 3' untranslated region of the histone mRNA" does appear in the specification and no new matter is added by the amendment.

## **II. The Claims Are Clear And Definite**

Claims 35-41 and 43-50 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. The Office Action asserts that the phrase "said target nucleic acid" can still be found in claims 35, 43 and 51. Applicants intended to amend each of claims 35-41 and 43-50 to recite "RNA of a selected organism" in place of "target nucleic acid" in Applicants' Amendment and Request for Reconsideration, dated July 24, 2000. However, as pointed out by the Office Action, Applicants inadvertently neglected to amend all of the occurrences of the phrase. Claims 35, 43 and 51 have been amended accordingly. In view of the amendment to the claims, Applicants respectfully request that the rejection under 35 U.S.C. § 112, second paragraph be withdrawn.

### III. The Claimed Invention Is Novel and Not Obvious

#### A. The Williams Reference

Claims 35-41 and 43-51 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Williams *et al.*, *Nuc. Acids. Res.*, **1994**, 22, 4660-4666 (hereinafter, the “Williams reference”). According to the Office Action, the histone mRNA disclosed in the Williams reference meets every element of these claims. Applicants traverse the rejection and respectfully request reconsideration because the Williams reference does not teach or suggest at least two aspects of Applicants’ claimed invention.

First, the Williams reference fails to teach or suggest an oligonucleotide that modulates the expression of RNA in a selected organism. Rather, the Williams reference reports that the binding of a 45kD stem-loop binding protein (SLBP) to the 3’ end of histone mRNA may affect processing, transport, and degradation of histone mRNA. Applicants have defined “modulation” at page 10, line 28 of the specification to mean “augmenting-or-diminishing RNA activity or expression.” The rejected claims refer only to “modulation of expression” of RNA and do not include modulation of activities. Alterations to the processing, transport, and degradation properties of histone mRNA do not necessarily augment or diminish RNA expression, as recited in Applicants’ claims.

Second, as acknowledged in the Office Action mailed September 13, 2001, the molecular interaction site taught by the Williams reference is not identified in the manner recited in claims 35-41 and 43-51. The Office Action did not consider the identification aspect of Applicants’ invention as a limitation because it characterized Applicants’ claims as “product-by-process” claims. Applicants respectfully request reconsideration of this conclusion because Applicants’ claims are not “product-by-process” claims.

Product-by-process claims are used to describe a product “that resists definition by other than the process by which it is made.” *In re Thorpe*, 227 U.S.P.Q. 964, 966 (Fed. Cir. 1985). “That a process limitation appears in a claim does not convert it to a product by process claim.” *Fromson v. Advance Offset Plate, Inc.*, 219 U.S.P.Q. 1137, 1141 (Fed. Cir. 1983), see also *Biacore v. Thermo Bioanalysis Corp.*, 79 F. Supp. 2d 422, 456 (D. Del. 1999) (citing *In re Hughes*, 182 U.S.P.Q. 106, 108 (C.C.P.A. 1974); *In re Garnero*, 412 F.2d 276, 279 (C.C.P.A. 1969)) (“The mere use in a claim

of structural or characterizing terms derived from processes or methods ... does not prevent a claim from being considered a true product claim"). Applicants have described their claimed invention recited in claims 35-41 and 43-51 primarily in terms of structure or physical characteristics and not by the process by which the invention is made. Therefore, Applicants' claims are not true "product-by-process" claims. The process limitation recited in these claims, however, imparts patentability to the claimed compounds.

In view of the forgoing, Applicants respectfully request that the rejection under 35 U.S.C. § 102(b) be withdrawn.

### **B. The Garcia Reference**

Claims 27-29 and 35 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as allegedly being obvious over Garcia *et al.*, *J. Mol. Biol.*, **1995**, 254, 247-259 (hereinafter, the "Garcia reference"). Applicants traverse the rejection and respectfully request reconsideration because the Garcia reference does not teach or suggest every feature recited in the claims.

The Garcia reference fails to teach or suggest at least two aspects of applicants' claimed invention. First, the Garcia reference fails to teach or suggest an oligonucleotide, let alone an oligonucleotide that comprises a molecular interaction site, as recited in independent claims 27 and 35. Rather, the Garcia reference reports that the ribosome binding domain of *E. coli* translation initiation factor IF3 interacts with 16S RNA. However, the 16S RNA molecule that is relied on for the instant rejection is not an oligonucleotide, as called for by the claims. Applicants' claims clearly distinguish naturally-occurring nucleic acid molecules, such as prokaryotic RNA, from oligonucleotides. Indeed, one of ordinary skill in the art would understand that the oligonucleotides of the invention are prepared based on such RNA molecules. No such oligonucleotide is prepared in, or taught by, the Garcia reference and, thus, the reference does not anticipate the claimed invention.

The Garcia reference further fails to teach or suggest that the molecular interaction site of the 16S RNA is present in "at least one additional prokaryotic RNA," as recited in the claims. The

purported molecular interaction site disclosed in the Garcia reference is only disclosed with regard to the 16S RNA molecule, and not with any other RNA.

Finally, with respect to the alternative § 103(a) rejection, the Office Action fails to make out a *prima facie* showing of obviousness. In establishing a *prima facie* case of obviousness under 35 U.S.C. § 103, it is incumbent upon the Examiner to provide a reason why one of ordinary skill in the art would have been led to modify a prior art reference or to combine reference teachings to arrive at the claimed invention. *Ex parte Clapp*, 227 U.S.P.Q. 972 (Bd. Pat. App. Int. 1985). To this end, the requisite motivation must stem from some teaching, suggestion or inference in the prior art as a whole or from the knowledge generally available to one of ordinary skill in the art and not from appellants' disclosure. See, for example, *Uniroyal Inc. v. Rudkin-Wiley Corp.*, 5 U.S.P.Q.2d 1434 (Fed. Cir. 1988); and *Ex parte Nesbit*, 25 U.S.P.Q.2d 1817, 1819 (Bd. Pat. App. Int. 1992). Here, the Office Action fails to provide a reason as to why one of ordinary skill in the art would have been led to modify the ribosomal subunit of the Garcia reference to arrive at the oligonucleotide of claimed invention. Nor does the Office Action point to any reference that discloses the purported molecular interaction site of the ribosomal subunit is found in an additional prokaryotic RNA. Absent such teachings, the rejection of the claims as allegedly being obvious must be withdrawn.

In view of the foregoing, Applicants respectfully request that the rejection under 35 U.S.C. § 102(b) or, alternatively, 35 U.S.C. § 103(a) be withdrawn.

### C. The Gutell Reference

Claims 35-41 and 43-50 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as allegedly being obvious over Gutell *et al.*, *Nuc. Acids Res.*, **1993**, *21*, 3051-3054 (hereinafter, the "Gutell reference"). Applicants traverse the rejection and respectfully request reconsideration because the Gutell reference does not teach or suggest every feature recited in the claims.

The Gutell reference illustrates proposed secondary structure models for 16S and 16S-like rRNAs for *E. coli*, yeast, and *C. elegans*. The Office Action improperly asserts a scientific conclusion that is neither taught nor proposed in the Gutell reference. The Office Action asserts at

page 7 under point 8 that “In this study, they aligned 16S- and 16S-like ribosomal RNA sequences from . . . different organisms and . . . compared their structure second structure.” The Gutell reference clearly states the objectives of the subject paper at page 3051, right column under *Objectives*: “[P]resent our most current comparative interpretation of the *Escherica coli* 16S rRNA secondary structure.” Moreover the Gutell reference further states under *Objective 4* at page 3051 bottom of right column: “[I]llustrated in this article are three divergent 16S and 16S-like rRNAs structure models . . .” This article contradicts the assertions of the Office Action, at least because of that admission of the divergency of the secondary structures. Moreover, the Gutell reference further teaches away from the conclusions asserted in the Office Action at the footnote to Figure 2 wherein it is admitted that the sequence is modified. Additionally, only Figure 1 provides sequence numbering so that the reader may verify the graphical alignment. The Gutell reference further provides that the graphical structures were manipulated “manually,” through the use of an “interactive sequence alignment editor” to arrive at the depicted figures. Thus, the authors of the Gutell reference did not intend to study the potential alignment of the subject regions of rRNA across species, but rather intended to present “their” structure for the *Escherica coli* 16S rRNA with a non-comparative sampling of other secondary structures. For at least these reasons, the Gutell reference fails to teach at least one element of claim 42, in particular, “identifying at least one sequence region which is conserved among said plurality of nucleic acids and said RNA of a selected organism.”

In addition, as recognized in the Office Action, the Gutell reference does not identify a molecular interaction site or determine the secondary structure of a conserved region within the RNA. The Office Action mistakenly asserts, however, that these features are simply inherent limitations. As discussed in Applicants’ Amendment dated July 24, 2000, the Patent Office failed to offer evidence that the inherent characteristics would necessarily (*i.e.*, always) be present in an oligonucleotide prepared from the RNA of the Gutell reference. The instant Office Action also does not address the lack of evidence of record, and, accordingly, Applicants request that the rejection based on the Gutell reference be withdrawn.

Further, the ribosomal RNA of the Gutell reference does not anticipate the oligonucleotide called for in Applicants’ claims. The Gutell reference also fails to teach or suggest that the purported

molecular interaction site on the 16S structure is found in one additional procaryotic RNA. That the RNA disclosed in the Gutell reference can be found in "different organisms," as pointed out in the Office Action, is of no consequence, and fails to bring the teachings of the Gutell reference within the scope of Applicants' claims.

Finally, for the same reasons set forth above with respect to the Garcia reference, the Office Action fails to make out a *prima facie* case of obviousness. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 102(b) or, alternatively, 35 U.S.C. § 103(a) be withdrawn.

#### IV. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 564-8906 if there are any questions regarding Applicants' claimed invention. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Application:**

Pages 1-96 containing the Sequence Listing have been deleted.

**In the Specification:**

Paragraph beginning at page 5, line 21 of the specification has been deleted.

Paragraph beginning at page 5, line 23 of the specification has been deleted.

Paragraph beginning at page 5, line 25 of the specification has been deleted.

Paragraph beginning at page 5, line 28 of the specification has been amended as follows:

Figure 6 [9] shows an exemplary descriptor.

Paragraph beginning at page 5, line 29 of the specification has been amended as follows:

Figure 7 [10] shows a set of e-value scores for ferritin.

Paragraph beginning at page 6, line 1 of the specification has been deleted.

Paragraph beginning at page 6, line 3 of the specification has been deleted.

Paragraph beginning at page 6, line 5 of the specification has been amended as follows:

Figure 8 [13] shows a representative lookup table used in Q-compare or CompareOverWins.

Paragraph beginning at page 6, line 7 of the specification has been amended as follows:

Figure 9 [14] shows a representative block diagram of a program called RevComp.

Paragraph beginning at page 6, line 8 of the specification has been amended as follows:



Figure 10 [15] shows a representative flow chart showing preferred steps of a preferred database search strategy for ortholog finding.

Paragraph beginning at page 6, line 10 of the specification has been deleted.

Paragraph beginning at page 6, line 12 of the specification has been deleted.

Paragraph beginning at page 6, line 14 of the specification has been amended as follows:

Figure 11 [18] shows a representative flow scheme showing preferred steps for a preferred SEALS strategy.

Paragraph beginning at page 6, line 16 of the specification has been deleted.

Paragraph beginning at page 6, line 18 of the specification has been amended as follows:

Figure 12 [20] represents a genetic map showing a conserved iron response element in the 5' UTR of mouse and human ferritin.

Paragraph beginning at page 6, line 20 of the specification has been deleted.

Paragraph beginning at page 6, line 22 of the specification has been deleted.

Paragraph beginning at page 6, line 24 of the specification has been deleted.

Paragraph beginning at page 6, line 25 of the specification has been deleted.

Paragraph beginning at page 6, line 26 of the specification has been amended as follows:

Figure 13 [25] shows representative flow scheme showing preferred steps for a preferred Structure Predictor strategy.

Paragraph beginning at page 6, line 28 of the specification has been deleted.

Paragraph beginning at page 6, line 30 of the specification has been deleted.

Paragraph beginning at page 7, line 1 of the specification has been amended as follows:

Figure 14 [28] shows a representative structure drawing of ferritin 5' UTR.

Paragraph beginning at page 7, line 2 of the specification has been deleted.

Paragraph beginning at page 7, line 3 of the specification has been deleted.

Paragraph beginning at page 7, line 5 of the specification has been deleted.

Paragraph beginning at page 7, line 6 of the specification has been deleted.

Paragraph beginning at page 7, line 7 of the specification has been deleted.

Paragraph beginning at page 7, line 9 of the specification has been deleted.

Paragraph beginning at page 7, line 11 of the specification has been deleted.

Paragraph beginning at page 7, line 12 of the specification has been deleted.

Paragraph beginning at page 7, line 14 of the specification has been deleted.

Paragraph beginning at page 7, line 15 of the specification has been deleted.

Paragraph beginning at page 7, line 16 of the specification has been deleted.

Paragraph beginning at page 7, line 18 of the specification has been deleted.

Paragraph beginning at page 7, line 20 of the specification has been deleted.

Paragraph beginning at page 7, line 22 of the specification has been deleted.

Paragraph beginning at page 7, line 23 of the specification has been deleted.

Paragraph beginning at page 7, line 25 of the specification has been deleted.

Paragraph beginning at page 7, line 27 of the specification has been deleted.

Paragraph beginning at page 7, line 28 of the specification has been deleted.

Paragraph beginning at page 7, line 30 of the specification has been deleted.

Page 8 has been deleted.

Paragraph beginning at page 9, line 1 of the specification has been deleted.

Paragraph beginning at page 9, line 3 of the specification has been deleted.

Paragraph beginning at page 9, line 5 of the specification has been deleted.

Paragraph beginning at page 9, line 7 of the specification has been amended as follows:

Figure 15 [66] shows a representative mass-spec structure probe analysis of region 1 of ornithine decarboxylase 3' UTR.

Paragraph beginning at page 9, line 9 of the specification has been deleted.

Paragraph beginning at page 9, line 11 of the specification has been deleted.

Paragraph beginning at page 9, line 13 of the specification has been deleted.

Paragraph beginning at page 9, line 15 of the specification has been deleted.

Paragraph beginning at page 9, line 17 of the specification has been deleted.

Paragraph beginning at page 9, line 19 of the specification has been deleted.

Paragraph beginning at page 9, line 21 of the specification has been deleted.

Paragraph beginning at page 9, line 23 of the specification has been deleted.

Paragraph beginning at page 9, line 25 of the specification has been deleted.

Paragraph beginning at page 9, line 27 of the specification has been deleted.

Paragraph beginning at page 9, line 29 of the specification has been deleted.

Paragraph beginning at page 9, line 30 of the specification has been deleted.

Paragraph beginning at page 10, line 1 of the specification has been deleted.

Paragraph beginning at page 10, line 3 of the specification has been deleted.

Paragraph beginning at page 10, line 5 of the specification has been deleted.

Paragraph beginning at page 10, line 6 of the specification has been deleted.

Paragraph beginning at page 10, line 8 of the specification has been deleted.

Paragraph beginning at page 10, line 10 of the specification has been deleted.

Paragraph beginning at page 10, line 11 of the specification has been deleted.

Paragraph beginning at page 10, line 12 of the specification has been deleted.

Paragraph beginning at page 10, line 14 of the specification has been deleted.

Paragraph beginning at page 10, line 16 of the specification has been deleted.

Paragraph beginning at page 10, line 17 of the specification has been deleted.

Paragraph beginning at page 10, line 18 of the specification has been deleted.

Paragraph beginning at page 10, line 19 of the specification has been deleted.

Paragraph beginning at page 10, line 20 of the specification has been deleted.

Paragraph beginning at page 10, line 21 of the specification has been deleted.

Paragraph beginning at page 10, line 22 of the specification has been deleted.

Paragraph beginning at page 10, line 23 of the specification has been deleted.

Paragraph beginning at page 10, line 24 of the specification has been deleted.

Paragraph beginning at page 10, line 25 of the specification has been deleted.

Paragraph beginning at page 16, line 11 of the specification has been amended as follows:

Additional nucleic acid targets may be determined independently or can be selected from publicly available prokaryotic and eukaryotic genetic databases known to those skilled in the art. Preferred databases include, for example, Online Mendelian Inheritance in Man (OMIM), the Cancer Genome Anatomy Project (CGAP), GenBank, EMBL, PIR, SWISS-PROT, and the like. OMIM, which is a database of genetic mutations associated with disease, was developed, in part, for the National Center for Biotechnology Information (NCBI). OMIM is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/Omim/](http://ncbi.nlm.nih.gov/Omim/). [OMIM can be accessed through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/Omim/>.] CGAP, which is an interdisciplinary program to establish the information and technological tools required to decipher the molecular anatomy of a cancer cell. [CGAP can be accessed through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/ncicgap/>.] CGAP is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/ncicgap/](http://ncbi.nlm.nih.gov/ncicgap/). Some of these databases may contain complete or partial nucleotide sequences. In addition, nucleic acid targets can also be selected from private genetic databases. Alternatively, nucleic acid targets can be selected from available publications or can be determined especially for use in connection with the present invention.

Paragraph beginning at page 16, line 25 of the specification has been amended as follows:

After a nucleic acid target is selected or provided, the nucleotide sequence of the nucleic acid target is determined and then compared to the nucleotide sequences of a plurality of nucleic acids from different taxonomic species. In one embodiment of the invention, the nucleotide sequence of the nucleic acid target is determined by scanning at least one genetic database or is

identified in available publications. Preferred databases known and available to those skilled in the art include, for example, the Expressed Gene Anatomy Database (EGAD) and Unigene-Homo Sapiens database (Unigene), GenBank, and the like. EGAD contains a non-redundant set of human transcript (HT) sequences [and can be accessed through the Internet at, for example, <http://www.tigr.org/tdb/egad/egad.html>] and is publicly available through the Internet at the world wide web at, for example, [tigr.org/tdb/egad/egad.html](http://www.tigr.org/tdb/egad/egad.html). Unigene is a system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each Unigene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location.

Paragraph beginning at page 17, line 10 of the specification has been amended as follows:

In addition, Unigene contains hundreds of thousands of novel expressed sequence tag (EST) sequences. [Unigene can be accessed through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/UniGene/>.] Unigene is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/UniGene/](http://www.ncbi.nlm.nih.gov/UniGene/). These databases can be used in connection with searching programs such as, for example, Entrez, which is known and available to those skilled in the art, and the like. [Entrez can be accessed through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/Entrez/>.] Entrez is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/Entrez](http://www.ncbi.nlm.nih.gov/Entrez/). Preferably, the most complete nucleic acid sequence representation available from various databases is used. The GenBank database, which is known and available to those skilled in the art, can also be used to obtain the most complete nucleotide sequence. GenBank is the NIH genetic sequence database and is an annotated collection of all publicly available DNA sequences. GenBank is described in, for example, Nuc. Acids Res., 1998, 26, 1-7, which is incorporated herein by reference in its entirety, and can be accessed by those skilled in the art [through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html>] through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/Web/Genbank/index.html](http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html). Alternatively, partial nucleotide sequences of nucleic acid targets can be used when a complete nucleotide sequence is not available.

Paragraph beginning at page 17, line 25 of the specification has been amended as follows:

In another embodiment of the present invention, the nucleotide sequence of the nucleic acid target is determined by assembling a plurality of overlapping expressed sequence tags (ESTs). The EST database (dbEST), which is known and available to those skilled in the art, comprises approximately one million different human mRNA sequences comprising from about 500 to 1000 nucleotides, and various numbers of ESTs from a number of different organisms. [dbEST can be accessed through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/dbEST/index.html>.] dbEST is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/dbEST/index.html](http://www.ncbi.nlm.nih.gov/dbEST/index.html). These sequences are derived from a cloning strategy that uses cDNA expression clones for genome sequencing. ESTs have applications in the discovery of new genes, mapping of genomes, and identification of coding regions in genomic sequences. Another important feature of EST sequence information that is becoming rapidly available is tissue-specific gene expression data. This can be extremely useful in targeting selective gene(s) for therapeutic intervention. Since EST sequences are relatively short, they must be assembled in order to provide a complete sequence. Because every available clone is sequenced, it results in a number of overlapping regions being reported in the database.

Paragraph beginning at page 18, line 9 of the specification has been amended as follows:

Assembly of overlapping ESTs extended along both the 5' and 3' directions results in a full-length "virtual transcript." The resultant virtual transcript may represent an already characterized nucleic acid or may be a novel nucleic acid with no known biological function. The Institute for Genomic Research (TIGR) Human Genome Index (HGI) database, which is known and available to those skilled in the art, contains a list of human transcripts. [TIGR can be accessed through the Internet at, for example, <http://www.tigr.org/>.] TIGR is publicly available through the Internet at the world wide web at, for example, [tigr.org/](http://www.tigr.org/). The transcripts were generated in this manner using TIGR-Assembler, an engine to build virtual transcripts and which is known and available to those skilled in the art. TIGR-Assembler is a tool for assembling large sets of overlapping sequence data such as ESTs, BACs, or small genomes, and can be used to assemble eukaryotic or prokaryotic sequences.



TIGR-Assembler is described in, for example, Sutton, *et al.*, *Genome Science & Tech.*, **1995**, *1*, 9-19, which is incorporated herein by reference in its entirety, [and can be accessed through the Internet at, for example, [ftp://ftp.tigr.org/pub/software/TIGR\\_assembler](ftp://ftp.tigr.org/pub/software/TIGR_assembler)] and is publicly available through the Internet via file transfer program at, for example [tigr.org/pub/software/TIGRAssembler](http://tigr.org/pub/software/TIGRAssembler). In addition, GLAXO-MRC, which is known and available to those skilled in the art, is another protocol for constructing virtual transcripts. In addition, "Find Neighbors and Assemble EST Blast" protocol, which runs on a UNIX platform, has been developed by Applicants to construct virtual transcripts. Preferred steps in the Find Neighbors and Assemble EST Blast protocol is described in the flowchart set forth in Figure 2. PHRAP is used for sequence assembly within Find Neighbors and Assemble EST Blast. [PHRAP can be accessed through the Internet at, for example, <http://chimera.biotech.washington.edu/uwgc/tools/phrap.htm>.] PHRAP is publicly available through the Internet at, for example, [chimera.biotech.washington.edu/uwgc/tools/phrap.htm](http://chimera.biotech.washington.edu/uwgc/tools/phrap.htm). One skilled in the art can construct source code to carry out the preferred steps set forth in Figure 2.

Paragraph beginning at page 19, line 23 of the specification has been amended as follows:

Sequence similarity searches can be performed manually or by using several available computer programs known to those skilled in the art. Preferably, Blast and Smith-Waterman algorithms, which are available and known to those skilled in the art, and the like can be used. Blast is NCBI's sequence similarity search tool designed to support analysis of nucleotide and protein sequence databases. [Blast can be accessed through the Internet at, for example, <http://www.ncbi.nlm.nih.gov/BLAST/>.] Blast is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/). The GCG Package provides a local version of Blast that can be used either with public domain databases or with any locally available searchable database. GCG Package v.9.0 is a commercially available software package that contains over 100 interrelated software programs that enables analysis of sequences by editing, mapping, comparing and aligning them. Other programs included in the GCG Package include, for example, programs which facilitate RNA secondary structure predictions, nucleic acid fragment assembly, and evolutionary analysis. In addition, the most prominent genetic databases (GenBank, EMBL, PIR,

and SWISS-PROT) are distributed along with the GCG Package and are fully accessible with the database searching and manipulation programs. [GCG can be accessed through the Internet at, for example, <http://www.gcg.com/>.] GCG is publicly available through the Internet at the world wide web at, for example, [gcg.com/](http://www.gcg.com/). Fetch is a tool available in GCG that can get annotated GenBank records based on accession numbers and is similar to Entrez. Another sequence similarity search can be performed with GeneWorld and GeneThesaurus from Pangea. GeneWorld 2.5 is an automated, flexible, high-throughput application for analysis of polynucleotide and protein sequences. GeneWorld allows for automatic analysis and annotations of sequences. Like GCG, GeneWorld incorporates several tools for homology searching, gene finding, multiple sequence alignment, secondary structure prediction, and motif identification. GeneThesaurus 1.0tm is a sequence and annotation data subscription service providing information from multiple sources, providing a relational data model for public and local data.

Paragraph beginning at page 20, line 24 of the specification has been amended as follows:

Another toolkit capable of doing sequence similarity searching and data manipulation is SEALS, also from NCBI. This tool set is written in perl and C and can run on any computer platform that supports these languages. [It is available for download, for example, at: <http://www.ncbi.nlm.nih.gov/Walker/SEALS/>.] It is publicly available through the Internet at the world wide web at, for example, [ncbi.nlm.nih.gov/Walker/SEALS/](http://www.ncbi.nlm.nih.gov/Walker/SEALS/). This toolkit provides access to Blast2 or gapped blast. It also includes a tool called tax\_collector which, in conjunction with a tool called tax\_break, parses the output of Blast2 and returns the identifier of the sequence most homologous to the query sequence for each species present. Another useful tool is feature2fasta which extracts sequence fragments from an input sequence based on the annotation. An exemplary use for this tool is to create sequence files containing the 5' untranslated region of a cDNA sequence.

Paragraph beginning at page 21, line 30 of the specification has been amended as follows:

In another embodiment of the invention, the sequences required are obtained by searching ortholog databases. One such database is Hovergen, which is a curated database of vertebrate

orthologs. Ortholog sets may be exported from this database and used as is, or used as seeds for further sequence similarity searches as described above. Further searches may be desired, for example, to find invertebrate orthologs. [Hovergen can be downloaded, for example, at: <ftp://pbil.univ-lyon1.fr/pub/hovergen/>.] Hovergen is publicly available through the Internet via file transfer program at, for example, [pbil.univ-lyon1.fr/pub/hovergen/](ftp://pbil.univ-lyon1.fr/pub/hovergen/). A database of prokaryotic orthologs, COGS, is available and can be used interactively through the Internet at the world wide web at, for example, [on the internet, for example at: <http://www.ncbi.nlm.nih.gov/COG/>.

Paragraph beginning at page 23, line 22 of the specification has been amended as follows:

Sequence homology between the window sequence of the target nucleic acid and the query sequence of any of the plurality of nucleic acid sequences obtained as described above, is preferably at least 60%, more preferably at least 70%, more preferably at least 80%, and most preferably at least 90%. The most preferable method of choosing the threshold is to have the computer automatically try all thresholds from 50% to 100% and choose a threshold based a metric provided by the user. One such metric is to pick the threshold such that exactly n hits are returned, where n is usually set to 3. This process is repeated until every base on the query nucleic acid, which is a member of the plurality of nucleic acids described above, has been compared to every base on the master target sequence. The resulting scoring matrix can be plotted as a scatter plot. Based on the match density at a given location, there may be no dots, isolated dots, or a set of dots so close together that they appear as a line. The presence of lines, however small, indicates primary sequence homology. [A representative scatter plot of such interspecies sequence comparison is depicted in Figure 6.] Sequence conservation within nucleic acid molecules, particularly the UTRs of RNA, in divergent species is likely to be an indicator of conserved regulatory elements that are also likely to have a secondary structure. The results of the interspecies sequence comparison can be analyzed using MS Excel and visual basic tools in an entirely automated manner as known to those skilled in the art.

Paragraph beginning at page 23, line 22 of the specification has been amended as follows:

In another embodiment of the invention, secondary structure analysis is performed by self complementarity comparison. Preferably, self complementarity comparison is performed using Compare, described above. More preferably, Compare can be modified to expand the pairing matrix to account for G-U or U-G basepairs in addition to the conventional Watson-Crick G-C/C-G or A-U/U-A pairs. Such a modified Compare program (modified Compare) begins by predicting all possible base-pairings within a given sequence. As described above, a small but conserved region, preferably a UTR, is identified based on primary sequence comparison of a series of orthologs. In modified Compare, each of these sequences is compared to its own reverse complement. [Figure 7 depicts an exemplary self complementarity analysis.] Allowable base-pairings include Watson-Crick A-U, G-C pairing and non-canonical G-U pairing. An overlay of such self complementarity plots of all available orthologs, and selection for the most repetitive pattern in each, results in a minimal number of possible folded configurations. [Figure 8 shows an exemplary overlay.] These overlays can then be used in conjunction with additional constraints, including those imposed by energy considerations described above, to deduce the most likely secondary structure.

Paragraph beginning at page 24, line 17 of the specification has been amended as follows:

In one embodiment of the invention, secondary structure analysis is performed by alignment and covariance analysis. Numerous protocols for alignment and covariance analysis are known to those skilled in the art. Preferably, alignment is performed by ClustalW, which is available and known to those skilled in the art. ClustalW is a tool for multiple sequence alignment that, although not a part of GCG, can be added as an extension of the existing GCG tool set and used with local sequences. [ClustalW can be accessed through the Internet at, for example, <http://dot.imgen.bcm.tmc.edu:9331/multi-align/Options/clustalw.html>.] ClustalW is publicly available through the Internet at, for example, dot.imgen.bcm.tmc.edu:9331/multialign/Options/clustalw.html. ClustalW is also described in Thompson, *et al.*, *Nuc. Acids Res.*, **1994**, 22, 4673-4680, which is incorporated herein by reference in its entirety. These processes can be scripted to automatically use conserved UTR regions identified in earlier steps. Seqed, a UNIX command line interface available and known to those skilled in the art,

allows extraction of selected local regions from a larger sequence. Multiple sequences from many different species can be clustered and aligned for further analysis.

Paragraph beginning at page 25, line 11 of the specification has been amended as follows:

Covariation is a process of using phylogenetic analysis of primary sequence information for consensus secondary structure prediction. Covariation is described in the following references, each of which is incorporated herein by reference in their entirety: Gutell, *et al.*, "Comparative Sequence Analysis Of Experiments Performed During Evolution" In Ribosomal RNA Group I Introns, Green, Ed., Austin:Landes, **1996**; Gautheret, *et al.*, *Nuc. Acids Res.*, **1997**, 25, 1559-1564; Gautheret, *et al.*, *RNA*, **1995**, 1, 807-814; Lodmell, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1995**, 92, 10555-10559; Gautheret, *et al.*, *J. Mol. Biol.*, **1995**, 248, 27-43; Gutell, *Nuc. Acids Res.*, **1994**, 22, 3502-3517; Gutell, *Nuc. Acids Res.*, **1993**, 21, 3055-3074; Gutell, *Nuc. Acids Res.*, **1993**, 21, 3051-3054; Woese, *Proc. Natl. Acad. Sci. USA*, **1989**, 86, 3119-3122; and Woese, *et al.*, *Nuc. Acids Res.*, **1980**, 8, 2275-2293. Preferably, covariance software is used for covariance analysis. Preferably, Covariation, a set of programs for the comparative analysis of RNA structure from sequence alignments, is used. Covariation uses phylogenetic analysis of primary sequence information for consensus secondary structure prediction. [Covariation can be obtained through the Internet at, for example, <http://www.mbio.ncsu.edu/RNaseP/info/programs/programs.html>.] Covariation is publicly available through the Internet at the world wide web at, for example [mbio.ncsu.edu/RNaseP/info/programs/programs.html](http://www.mbio.ncsu.edu/RNaseP/info/programs/programs.html). A complete description of a version of the program has been published (Brown, J. W. 1991 Phylogenetic analysis of RNA structure on the Macintosh computer. CABIOS7:391-393). The current version is v4.1, which can perform various types of covariation analysis from RNA sequence alignments, including standard covariation analysis, the identification of compensatory base-changes, and mutual information analysis. The program is well-documented and comes with extensive example files. It is compiled as a stand-alone program; it does not require Hypercard (although a much smaller 'stack' version is included). This program will run in any Macintosh environment running MacOS v7.1 or higher. Faster processor machines (68040 or PowerPC) is suggested for mutual information analysis or the analysis of large

sequence alignments.

Paragraph beginning at page 26, line 5 of the specification has been amended as follows:

In another embodiment of the invention, secondary structure analysis is performed by secondary structure prediction. There are a number of algorithms that predict RNA secondary structures based on thermodynamic parameters and energy calculations. Preferably, secondary structure prediction is performed using either M-fold or RNA Structure 2.52. [M-fold can be accessed through the Internet at, for example, <http://www.ibc.wustl.edu/-zucker/ma/form2.cgi>] M-fold is publicly available through the Internet at the world wide web at, for example, [ibc.wustl.edu/-zucker/ma/form2.cgi](http://www.ibc.wustl.edu/-zucker/ma/form2.cgi) or can be downloaded for local use on UNIX platforms. M-fold is also available as a part of GCG package. RNA Structure 2.52 is a windows adaptation of the M-fold algorithm and [can be accessed through the Internet at, for example, <http://128.151.176.70/RNAstructure.html>] is publicly available through the Internet at, for example, [128.151.176.70/RNAstructure.html](http://128.151.176.70/RNAstructure.html).

Paragraph beginning at page 26, line 29 of the specification has been amended as follows:

In another preferred embodiment of the invention, the output of AlignHits is read by a program called RevComp. A block diagram of this program is shown in Figure 9 [14]. This program could be reproduced by one skilled in the art. A preferred purpose of this program is to use base pairing rules and ortholog evolution to predict RNA secondary structure. RNA secondary structures are composed of single stranded regions and base paired regions, called stems. Since structure conserved by evolution is searched, the most probable stem for a given alignment of ortholog sequences is the one which could be formed by the most sequences. Possible stem formation or base pairing rules is determined by, for example, analyzing base pairing statistics of stems which have been determined by other techniques such as NMR. The output of RevComp is a sorted list of possible structures, ranked by the percentage of ortholog set member sequences which could form this structure. Because this approach uses a percentage threshold approach, it is insensitive to noise sequences. Noise sequences are those that either not true orthologs, or sequences that made it into the output of AlignHits due to high sequence homology even though they do not represent an

example of the structure which is searched. A very similar algorithm is implemented using Visual basic for Applications (VBA) and Microsoft Excel to be run on PCs, to generate the reverse complement matrix view for the given set of sequences.

Paragraph beginning at page 28, line 13 of the specification has been amended as follows:

After the hypothetical structure motifs are determined from the secondary structure analysis described above, a family of structure descriptor elements is constructed. Preferably, the structural motifs described above are converted into a family of descriptor elements. An exemplary descriptor element is shown in Figure 6 [9]. One skilled in the art is familiar with construction of descriptors. Structure descriptors are described in, for example, Laferriere, et al., Comput. Appl. Biosci., 1994, 10, 211-212, incorporated herein by reference in its entirety. A different structure descriptor element is constructed for each of the structural motifs identified from the secondary structure analysis. Briefly, the secondary structure is converted to a generic text string, such as shown in Figure 6 [9]. For novel motifs, further biochemical analysis such as chemical mapping or mutagenesis may be needed to confirm structure predictions. Descriptor elements may be defined to have various stringency.

Paragraph beginning at page 28, line 24 of the specification has been amended as follows:

For example, referring to Figure 6 [9], the region termed H1, which comprises the first region of the stem, can be described as NNN:NNN, which contemplates any complementary base pairing including G-C, C-G, A-U, and U-A. The H1 region may also be designated so as to include only C-G or A-U, etc., base pairing. In addition, the descriptor elements can be defined to allow for a wobble. Thus, descriptor elements can be defined to have any level of stringency desired by the user. Applicants' invention, thus, is also directed to a database comprising different descriptor elements.

Paragraph beginning at page 29, line 10 of the specification has been amended as follows:

In one embodiment of the invention, nucleic acids having secondary structure which correspond to the structure descriptor elements are identified by searching at least one database. Any

genetic database can be searched. Preferably, the database is a UTR database, which is a compilation of the untranslated regions in messenger RNAs. A UTR database [is accessible through the Internet at, for example, <ftp://area.ba.cnr.it/pub/embnet/database/utr/>] is publicly available through the Internet via file transfer program at [area.ba.cnr.it/pub/embnet/database/utr/](http://area.ba.cnr.it/pub/embnet/database/utr/). Preferably the database is searched using a computer program, such as, for example, Rnamot, a UNIX-based motif searching tool available from Daniel Gautheret. Each "new" sequence that has the same motif is then queried against public domain databases to identify additional sequences. Results are analyzed for recurrence of pattern in UTRs of these additional ortholog sequences, as described below, and a database of RNA secondary structures is built. One skilled in the art is familiar with Rnamot. Briefly, Rnamot takes a descriptor string, such as the one shown in Figure 6 [9], and searches any Fasta format database for possible matches. Descriptors can be very specific, to match exact nucleotide(s), or can have built-in degeneracy. Lengths of the stem and loop can also be specified. Single stranded loop regions can have a variable length. G-U pairings are allowed and can be specified as a wobble parameter. Allowable mismatches can also be included in the descriptor definition. Functional significance is assigned to the motifs if their biological role is known based on previous analysis. Known regulatory regions such as Iron Response Element have been found using this technique (see, Example 1 below). In embodiments of the invention in which a database containing prokaryotic molecular interaction sites is compiled, it is preferable to refrain from searching human sequences or, alternatively, discarding human sequences when found.

Paragraph beginning at page 32, line 26 of the specification has been amended as follows:

An early step in the process is to use the master sequence (nucleotide or protein) to find and rank related sequences in the database (orthologs and paralogs). Sequence similarity search algorithms are used for this purpose. All sequence similarity algorithms calculate a quantitative measure of similarity for each result compared with the master sequence. An example of a quantitative result is an E-value obtained from the Blast algorithm. The E-values for a blast search of the non-redundant GenBank database using ferritin mRNA as the query sequence illustrates the use of quantitative analysis of sequence similarity searches. The E-value is the probability that a match



between a query sequence and a database sequence occurs due to random chance. Therefore, the lower an E-value the more likely that two sequences are truly related. A plot of the lowest E-value scores for ferritin is shown in Figure 7 [10]. Sequences that meet the cutoff criteria are selected for more detailed comparisons according to a set of rules described below. Since an objective of the sequence similarity search to find distantly related orthologs and paralogs, it is preferable that the cutoff criteria not be too stringent, or the target of the search may be excluded.

Paragraph beginning at page 33, line 23 of the specification has been amended as follows:

When the human mRNA and mouse mRNA sequences for ferritin, which each contain an IRE in the 5'-UTR, are analyzed in this manner, a plot showing the regions of sequence similarity is produced[, as shown in Figure 19]. Pairwise analysis of the human and mouse ferritin mRNA sequences illustrate several important aspects of this type of analysis. Regions of each mRNA that encode the amino acid sequence have the highest degree of similarity, while the untranslated regions are less similar. [In Figure 19, the location of the IRE is indicated.] In both the human and mouse ferritin mRNAs the IREs are located in the extreme 5' end of each mRNA. This demonstrates an important point -- the sequence conservation in the region of the IRE structure does not stand out against the background of sequence similarity between the human and mouse ferritin sequences. In contrast, in the comparison of human and trout [(Figure 11)] or human and chicken [(Figure 12)] ferritin mRNAs, the IREs can be immediately identified. This is because the sequence of the UTRs between human and trout or human and chicken are separated by greater evolutionarily distance than human and mouse, which is logical in view of the evolutionary distance that separates humans from birds and fish compared with other mammals. Comparing the human sequence to that of birds and fish is informative because the natural drift due to evolution has allowed many sequence changes in the UTRs. However, the IRE sequences are more constrained because they form an important structure. Thus, they stand out better and can be more readily identified.

Paragraph beginning at page 34, line 28 of the specification has been amended as follows:

The software used in the present invention makes the decision whether or not to compare sequences pairwise using a lookup table based upon the evolutionary distances between species. An example of a small lookup table using the examples described above is shown in Figure 8 [13]. The lookup table in the present invention includes all species that have sequences deposited in GenBank. Q-Compare in conjunction with CompareOverWins decides which sequences to compare pairwise.

Paragraph beginning at page 35, line 4 of the specification has been amended as follows:

Sets of sequences that show evidence of conservation in orthologs and paralogs or other related genes are analyzed for the ability to form internal structure. This is accomplished by analyzing each sequence in a matrix where the sequence is plotted 5' to 3' on the X axis and its reverse complement is plotted 5' to 3' on the Y axis, such as in, for example, self-complementary analysis. Matches that correspond to potential intramolecular base pairs are scored according to a table of values. When the human ferritin IRE sequence is analyzed in this fashion, the diagonals indicate potential self-complementary regions. Each of the 13 IRE sequences described in this example were analyzed in the same fashion. While each of the sequences can form a variety of different structures, the structure most likely to occur is one common to all the sequences. By superimposing the plots of all 13 individual sequences [(see, Figure 8)], the potential structure common to all the sequences is deduced.

Paragraph beginning at page 36, line 5 of the specification has been amended as follows:

[The use of] Hovergen was used to identify related sequences at the species and order levels [is shown in Figure 16 (tree classification at the species level) and Figure 17 (classification at the order level)]. Sequences corresponding to each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding region was extracted using SEALS and COWX, as shown in Figure 11 [18].

Paragraph beginning at page 36, line 11 of the specification has been amended as follows:

The IRE sequences are more constrained because they form an important structure. Thus, they stand out better and can be more readily identified even in closely related sequences. However, for this to work for any gene, the compare algorithm has been rewritten (see, Figures 5A-C). This new tool, CompareOverWins, allows a dynamic selection of both the range of window sizes, as well the hit threshold. This algorithm needs as its input parsed and separated 5' and 3' UTR sequences. We use tools available within the Seals genome analysis package described earlier to achieve this. Figure 11 [18] describes the steps involved.

Paragraph beginning at page 36, line 19 of the specification has been amended as follows:

To identify the IRE [iron responseve element] using the methods described herein, the compare over windows [widows] algorithm was used and the results visualized using AlignHits (Figure 5D for the algorithm). [Representative results are shown in Figure 23.] In addition to optimizing the thresholding, CompareOverWins also extracts the sequence corresponding to the hits. ClustalW (version 1.74) was used on the extracted sequences to create a locally gapped alignment [(see, Figure 24)]. A representative flow scheme for this approach is shown in Figure 13 [25].

Paragraph beginning at page 36, line 27 of the specification has been amended as follows:

Sets of sequences that show evidence of conservation in orthologs and paralogs or other related genes are analyzed for the ability to form internal structure. This is accomplished by analyzing each sequence in a matrix where the sequeunce is plotted 5' to 3' on the X axis and its complement is plotted 5' to 3' on the Y axis, such as in, for example, self-complementary analysis. Matches that correspond to potential intramolecular base pairs are scored according to a table of values. When the human ferritin IRE sequence is analyzed in this fashion, the diagonals indicate potential self- complementary regions. Each of the 13 IRE sequences described in this example were analyzed in the same fashion. While each of the sequences can form a variety of different structures, the structure most likely to occur is one common to all the sequences. By superimposing the plots of all 13 individual sequences [(see, Figure 26)], the potential structure common to all the sequences is deduced.

Paragraph beginning at page 37, line 9 of the specification has been amended as follows:

The above scheme has been implemented algorithmically into a program called RevComp (*see*, Figure 9 [14]). RevComp creates a sorted list of all the structures. Representative results can be viewed either as a “dome” output [(*see*, Figure 27)] or as a “connect” or “ct” file which can be used in one of many RNA structure viewing programs (RNAstructure, RNAViz, etc.). A representative example of such a structure drawing is shown in Figure 14 [28].

Paragraph beginning at page 37, line 21 of the specification has been amended as follows:

Phylogenetic [Figures 29 and 30 represent phylogenetic] tree outputs for all Histone orthologs in Hovergen database was obtained. Each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding regions were extracted and compared using SEALS and COWX as described earlier (*see*, Figures 11 [18] and 13 [25]).

Paragraph beginning at page 37, line 26 of the specification has been amended as follows:

Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions [(*see*, Figure 31). One such region is shown encircled]. The sequences corresponding to the region of interest was extracted from all species for alignment with CLUSTAL W (1.74). Following extraction of sequence information from Align Hits, CLUSTAL W (1.74) was used to provide multiple sequence alignment shown [(*see*, Figure 32)]. Each of the putative hit sequences was analyzed for the ability to form internal structure. This was accomplished by analyzing each sequence in a matrix where the sequence was plotted 5' to 3' on the X axis and its complement is plotted 5' to 3' on the Y axis. Base-pairs along the diagonals indicate potential self-complementary regions that can form secondary structures. [Figure 33 shows a representative reverse complement matrix. Figure 34 shows a] A representative sequence alignment in a dome format can show [showing] potential stem formation between the base pairs. Following conversion of the dome format file to a ct file, RNA Structure 3.21 is used to visualize the structure [(*see*, Figure 35)].

Paragraph beginning at page 38, line 10 of the specification has been amended as follows:

Vimentin is an intermediate filament protein whose 3'UTR is highly conserved between species. Previous studies by Zehner *et al.*, (Nuc. Acids Res., 1997, 25, 3362-3370) has shown that a proposed a complex stem-loop structure contained within this region may be important for vimentin mRNA functions such as mRNA localization. The same region was identified using the present analysis, thus validating the present approach. In addition, based on the analyses described herein, a second stem-loop structure that occurs downstream of the previously proposed structure that may have a role in regulating vimentin fuction as well has been identified [(*see*, Figure 36)].

Paragraph beginning at page 38, line 18 of the specification has been amended as follows:

A representative phylogenetic tree output for all Vimentin orthologs in Hovergen database was obtained [is shown in Figure 37]. Each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding regions were extracted and compared using SEALS and COWX as described earlier (*see*, Figures 11 [18] and 13 [25]).

Paragraph beginning at page 38, line 23 of the specification has been amended as follows:

Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions. Two such regions appeared, and were used for subsequent analyses [(*see*, Figure 38)]. Following extraction of sequence information from Align Hits for the first region [1], CLUSTAL W was used to provide multiple sequence alignment [shown (*see*, Figure 39)]. Potential stem formation between base pairs was [is] given above the sequence alignment in a dome format [is shown in Figure 40.] Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure [(*see*, Figure 41)]. This structure is very similar to the one proposed by Zehner *et al.* [(*see*, Figure 42)]. Zehner *et al.* presented a detailed chemical analysis of their proposed structure for the minimal binding domain in the 3' UTR of Vimentin. This analysis included cleavage with single-strand-specific (ChS or T1) or double-strand-specific (V1) nucleases as well as after exposure to lead acetate.

Paragraph beginning at page 39, line 5 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the second region [2], CLUSTAL W was used to provide multiple sequence alignment [shown in Figure 43]. The potential stem formation between base pairs in the second region [2] was [is] given above the sequence alignment in a dome format [(see, Figure 44)]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure for the second region [2 (see, Figure 36)].

Paragraph beginning at page 39, line 18 of the specification has been amended as follows:

A representative phylogenetic tree output for all Transferrin receptor orthologs in Hovergen database was obtained [is shown in Figure 45]. Each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding region were extracted and compared using SEALS and COWX as described earlier (see, Figures 11 [18] and 13 [25]).

Paragraph beginning at page 39, line 23 of the specification has been amended as follows:

Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions, [as shown in Figure 46. This can be seen where a vertical line intersects a series of horizontal lines representing sequence information from a set of species. This] The first region, between base pairs 920 to 990, in the 3 prime UTR of transferrin receptor was extracted from all species for alignment with CLUSTAL W (1.74).

Paragraph beginning at page 39, line 28 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the first region [1], CLUSTAL W (1.74) was used to provide multiple sequence alignment [as shown in Figure 47]. A representative potential stem formation between base pairs was [is] given above the sequence alignment in a dome format [as shown in Figure 48]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure, [(see, Figure 49). This can be seen where a vertical line intersects a series of horizontal lines representing sequence information

from a set of species. This] The second region, between base pairs 990 to 1050, in the 3 prime UTR of transferrin receptor was extracted from all species for alignment with CLUSTAL W (1.74) [(see, Figure 50)].

Paragraph beginning at page 40, line 7 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the second region [2], CLUSTAL W (1.74) was used to provide multiple sequence alignment [as shown in Figure 51]. Potential stem formation between base pairs was [is] given above the sequence alignment in a dome format [as shown in Figure 52]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure [as shown in Figure 53]. Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions. [This can be seen where a vertical line intersects a series of horizontal lines representing sequence information from a set of species. This] The third region, between base pairs 1372 to 1423, in the 3 prime UTR of transferrin receptor was extracted from all species for alignment with CLUSTAL W (1.74) [(see, Figure 54)].

Paragraph beginning at page 40, line 17 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the third region [3], CLUSTAL W (1.Ex.34) was used to provide multiple sequence alignment [as shown in Figure 55]. Potential stem formation between base pairs was [is] given above the sequence alignment in a dome format [as shown in Figure 56]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure [as shown in Figure 57]. Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions. [This can be seen where a vertical line intersects a series of horizontal lines representing sequence information from a set of species. This] The fourth region, between base pairs 1439 to 1479, in the 3 prime UTR of transferrin receptor was extracted from all species for alignment with CLUSTAL W (1.74) [(see, Figure 58)].

Paragraph beginning at page 40, line 27 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the fourth region [4], CLUSTAL W (1.Ex.34) was used to provide multiple sequence alignment [as shown in Figure 59]. Potential stem formation between base pairs was [is] given above the sequence alignment in a dome format [is shown in Figure 60]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure [as shown in Figure 61]. Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions. [This can be seen where a vertical line intersects a series of horizontal lines representing sequence information from a set of species. This] The fifth region, between base pairs 1479 to 1542, in the 3 prime UTR of transferrin receptor was extracted from all species for alignment with CLUSTAL W (1.74) [(see, Figure 62)].

Paragraph beginning at page 41, line 6 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the fifth region [5], CLUSTAL W (1.Ex.34) was used to provide multiple sequence alignment [as shown in Figure 63]. Potential stem formation between base pairs was [is] given above the sequence alignment in a dome format [is shown in Figure 64]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure [as shown in Figure 65].

Paragraph beginning at page 41, line 12 of the specification has been amended as follows:

Ornithine decarboxylase (ODC) is the first enzyme in the polyamine biosynthetic pathway. Studies have shown existence of translational regulatory elements both in the 5' and 3' untranslated regions (Grens et al., J. Biol. Chem., 1990, 265, 11810). Secondary structures have been proposed to exist in both these regions, though there is no conclusive evidence for it. The methods described herein identified two structures in the 3' UTR, as shown below. The presence of one of these structures (see, Figure 15 [66]) was verified using mass spectrometry probing (Griffey, et al., Proc. SPIE-Int. Soc. Opt. Eng., 2985 (Ultrasensitive Biochemical Diagnostics II): 82-86, which is incorporated herein by reference in its entirety). Two representative sequences that showed slight



variation in their lengths [(see, Figure 67)] were made into RNA and subjected to MS structure probing. Results shown in Figure 15 [66] confirm the presence of a stem-loop structure. Accordingly, identification of a novel secondary structure can be identified from the methods described herein, and such existence has been independently verified by structure probing.

Paragraph beginning at page 41, line 25 of the specification has been amended as follows:

Phylogenetic tree outputs for all Ornithine Decarboxylase orthologs in Hovergen database were obtained [is shown in Figure 68 and Figure 69]. Each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding region were extracted and compared using SEALS and COWX as described earlier (see, Figures 11 [18] and 13 [25]).

Paragraph beginning at page 42, line 1 of the specification has been amended as follows:

Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions [as shown in Figure 70]. Two such regions appeared [appear], and were used for subsequent analyses. Following extraction of sequence information from the first region [1], CLUSTAL W (1.74) was used to provide multiple sequence alignment shown. Each of the putative hit sequences was analyzed for the ability to form internal structure [as shown] in a [the] reverse complement matrix [depicted in Figure 71]. This was accomplished by analyzing each sequence in a matrix where the sequence is plotted 5' to 3' on the X axis and its complement is plotted 5' to 3' on the Y axis. Base-pairs along the diagonals indicate potential self- complementary regions that can form secondary structures. Domes view of the potential stem formation between base pairs in region 1 is given above the sequence alignment was determined using RevComp [(see, Figure 72)]. RNA Structure 3.2 was used to visualize the structure [(see, Figure 73)].

Paragraph beginning at page 42, line 13 of the specification has been amended as follows:

Mass spectrometry analyses techniques were used to probe for structure. The cluster alignment of the first region of ornithine decarboxylase 3' UTR [Figure 67] showed presence of gaps/inserts in the multiple alignment. Two representative RNAs (gi404561 and gi35135) from the

alignments [shown in Figure 67] were used for this experiment. Analysis of the pattern of induced fragmentation showed a very strong likelihood for base-pairing along the top half of the stem-loop structure [(shown inverted in the figure)]. This corresponds to bases 11-14 and 20-23 in 404561 or bases 8-11 and 18-21 in 35135. Bulged bases (G9 in 404561 or U22 in 35135) also showed characteristic fragmentation pattern. The bottom-half of the structure appeared to be less stable, and showed some fragmentation where our analyses had predicted base-pairing. This was particularly true in the sequence 35135. This region, however, has several contiguous A-U or G-U base-pairs which tend to be less stable, and therefore have a higher probability of fragmentation.

Paragraph beginning at page 42, line 24 of the specification has been amended as follows:

Following extraction of sequence information from Align Hits for the second region [2], CLUSTAL W was used to provide multiple sequence alignment [shown as shown in Figure 74]. Potential stem formation between base pairs in the second region [2] was [is] given above the sequence alignment in a dome format [as shown in Figure 75]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure for the second region [2 as shown in Figure 76].

Paragraph beginning at page 43, line 2 of the specification has been amended as follows:

A representative phylogenetic tree output for all IL-2 orthologs in Hovengen database was obtained [is shown in Figure 77]. Each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding region were extracted and compared using SEALS and COWX as described earlier (*see*, Figures 11 [18] and 13 [25]).

Paragraph beginning at page 43, line 7 of the specification has been amended as follows:

Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions in the 3'UTR region. Two such regions appear, and were used for subsequent analyses [(*see*, Figure 78)]. Following extraction of sequence information from

Align Hits for the first region [1], CLUSTAL W (1.74) was used to provide multiple sequence alignment [shown in Figure 79]. Domes view of the potential stem formation between base pairs in the first region [1 is] was given above the sequence alignment [was determined] using RevComp [(see, Figure 80)]. RNA Structure 3.2 was used to visualize the structure [as depicted in Figure 81]. Following extraction of sequence information from Align Hits for the second region [2], CLUSTAL W (1.74) was used to provide multiple sequence alignment [shown in Figure 82]. Potential stem formation between base pairs in the second region was [2 is] given above the sequence alignment in a dome format [as shown in Figure 83]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure for the second region [2 as shown in Figure 84].

Paragraph beginning at page 43, line 20 of the specification has been amended as follows:

In addition to the two regions described above, a third region, downstream of, and partially overlapping the second region [2], was identified using an alternate reference sequence (3087784.fa) [and is shown in Figure 85]. Following extraction of sequence information from Align Hits for this region, CLUSTAL W (1.74) was used to provide multiple sequence alignment [shown in Figure 86]. Potential stem formation between base pairs in the third region [3 is] was shown [in Figure 87] above the sequence alignment in a dome format. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure for the third region [3 (see, Figure 88)].

Paragraph beginning at page 43, line 29 of the specification has been amended as follows:

Representative phylogenetic tree output for all IL-4 orthologs in Hovergen database was obtained [is shown in Figure 89]. Each of these orthologs was saved in GenBank format and grouped together in a single data file. Untranslated regions in both the 5' and 3' flanks of the coding region were extracted and compared using SEALS and COWX as described earlier (see, Figures 11 [18] and 13 [25]).

Paragraph beginning at page 44, line 4 of the specification has been amended as follows:

Following extraction and comparison by SEALS and COWX, Align Hits was used to determine potentially interesting regions in the 5'UTR region [as shown in Figure 90]. Following extraction of sequence information from Align Hits for the above region, CLUSTAL W (1.74) was used to provide multiple sequence alignment [shown in Figure 91]. Domes view of the potential stem formation between base pairs in the region was [is] given above the sequence alignment [was determined] using RevComp [(see, Figure 92)]. RNA Structure 3.2 was used to visualize the structure [as shown in Figure 93].

Paragraph beginning at page 44, line 11 of the specification has been amended as follows:

[Figure 94 depicts a representative] Align Hits was used to view [of] hits in the 3'UTR region of IL-4. Following extraction of sequence information from Align Hits for the 3' UTR region, CLUSTAL W (1.74) was used to provide multiple sequence alignment [as shown in Figure 95]. Potential stem formation between base pairs in the second region [2 is] was given above the sequence alignment in a dome format [is shown in Figure 96]. Following conversion of the dome format file to a ct file, RNA Structure 3.21 was used to visualize the structure for the second region [2 (see, Figure 97)].

#### **In the Claims:**

New claims 52-67 have been added.

Claims 35, 43 and 51 have been amended as follows:

35. (Twice Amended) An oligonucleotide comprising a molecular interaction site that is present in the RNA of a selected organism and in the RNA of at least one additional organism, wherein said molecular interaction site serves as a binding site for at least one molecule that when bound to said molecular interaction site modulates the expression of said RNA in said selected organism, wherein said oligonucleotide does not comprise the iron response element, wherein said molecular interaction site is identified by a method comprising:

comparing the nucleotide sequence of said RNA of a selected organism with the nucleotide sequences of a plurality of nucleic acids from different taxonomic species;

identifying at least one sequence region which is conserved among said plurality of nucleic acids and said RNA of a selected organism [target nucleic acid];  
determining whether said conserved region has secondary structure; and  
for said conserved region having secondary structure, identifying said secondary structure.

43. (Amended) The oligonucleotide of claim 35 further comprising comparing said RNA of a selected organism [target nucleic acid] to paralogous nucleic acids.

51. (Amended) An oligonucleotide comprising a molecular interaction site that is present in the RNA of a selected organism and in the RNA of at least one additional organism, wherein said molecular interaction site serves as a binding site for at least one molecule that when bound to said molecular interaction site modulates the expression of said RNA in said selected organism, wherein said oligonucleotide does not comprise the iron response element, wherein said molecular interaction site is identified by a method comprising:

comparing the nucleotide sequence of said RNA of a selected organism [target nucleic acid] with the nucleotide sequences of a plurality of nucleic acids from different taxonomic species, wherein said nucleotide sequence of said RNA of a selected organism is determined by assembling a plurality of expressed sequence tags;

identifying at least one sequence region which is conserved among said plurality of nucleic acids and said RNA of a selected organism;

determining whether said conserved region has secondary structure; and

for said conserved region having secondary structure, identifying said secondary structure.